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TITLE

PROCESS FOR THE EXTRACTION OF CELL WALL COMPONENTS AND LESS ACCESSIBLE PROTEINS FROM CEREAL BRANS SUBSTANTIALLY FREE OF SOLUBLE COMPOUNDS

## DESCRIPTION

## TECHNICAL FIELD

The present invention relates to process for the extraction of cell wall components and less 10 accessible proteins from cereal brans substantially five of soluble compounds, the compounds thus recovered as well as their use.

#### INTRODUCTION

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Bran is defined as the seed coat of cereal grains such as wheat, barley, rye, triticale, oat or 15 rice. Anatomically, bran comprises the outer layers of the seed, known as the pericarp-testa and an inner layer known as the aleurone layer, which is often classified as the outermost layer of the endosperm. However, from the practical point of view cereal bran is herein defined as the remaining material after the conventional milling or polishing of cereal grains and contains primarily pericarp-testa and aleurone layer components, along with the cereal 20 germ and residual parts of the endosperm. The relative amounts of each component will depend upon the type of cereal and milling technique applied.

Within this definition, bran therefore contains all of the pericarp- testa components, the aleurone layer, the germ components including germ proteins and oils, along with a residual amount of endosperm starch, gluten and pentosans.

In this patent, the term cereal bran substantially free of soluble compounds or "cleaned bran" refers to any cereal bran, which has been processed, after conventional milling or polishing, by any means so as to remove substantial mounts of soluble components, which are extracted by water or organic solvents. The resulting material, hereafter referred to as cleaned bran, should contain rather limited amounts of soluble sugars, starch and gluten (less than 1%), but it may still contain some proteins and fats, which are less accessible

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and/or soluble. The cleaned bran consists primarily of cell wall components, of which hemicellulose is the most abundant.

- The invention relates to methods, procedures and an industrial process for the wetfractionation of bran into two protein rich fractions, one of which contains the germ oils and related components, a fibre fraction, which also retains most of the aleurone proteins, and a sugar syrup fraction.
- 10 The invention is centred around the wet-milling of than in the presence of enzymes: a) starch degrading enzymes (polysaccharidases) such as alpha amylases, and amyloglucosidases, and b) non-starch degrading enzymes (polysaccharidases) and c) a phytase, under appropriate conditions of temperature, i.e. from 50 to 90, more preferably from 50 to 75, and pH from 4 to 7.5. This is followed by the separation of the above listed components from aqueous suspension using mainly centrifugal separation methods. The pH when using an alpha amylase is normally around 7, and when using an amyloglucosidase it is around 4.5. The enzymes are used normally in a cocktail comprising 200 to 1500 IU/g of substrate.
- 20 Presently there exists no commercial method for well fractionating cereal bran to produce the specific products mentioned above.

This invention relates to methods, procedures and an industrial process for the wetfractionation of cleaned bran into one protein rich fraction, which contains proteins from the aleurone cells, a soluble hemicellulose fraction, a soluble oligosaccharide fraction and an insoluble fibre fraction.

This invention further aims at the fractionation of cleaned bran by combining wet-milling and enzymatic hydrolysis specifically with food grade xylanases under well-controlled conditions of temperature, i.e. from 35 to 80°C, more preferably from 40 to 50°C, and pH from 4 to 7, preferably 4.5 to 5.5.

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This step is followed by the separation of insoluble fibre and protein fractions from aqueous suspension using centrifugal separation methods while both hemicellulose and oligosaccharide fractions are separated by size exclusion techniques such as ultrafiltration.

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Presently there exist no commercial enzyme-based methods for wet-fractionating of cereal brans or derived products such as cleaned bran, which is capable of extracting high amounts of less accessible components, such as hemicellulose and aleurone proteins.

#### 10 PRIOR ART

US patent 4,361,651 describes a process for making fermentable sugars and high protein products from grain, mainly maize. In this method, grain is steeped for 10-30 hours, prior to milling and separation of the germ component, saccharification of carbohydrates (mainly starch), and separation of fibre. The yield of starch is maximised for fermentation to alcohol. Within the described process there is no specific fractionation of the bran component, separation of protein types or consideration of the germ component.

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US patent 5,312,636 informs on a process for fractionating crop into industrial raw material. This is focused on oat grain and incorporates bran fractionation procedures that involve the extraction of more hydrophobic comporants such as lipids in polar organic solvents prior to the alkaline extraction of residual bran to produce beta-glucan, protein and degummed fibres. The use of the organic solvent is alkey step in the process and hydrolysing enzymes are not utilised during the fractionation procedure.

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Two related US patents (4,171,383 and 4,171,384) inform on dry and wet milling procedures for refining whole wheat grain. US-A-4,471,383 focuses on wet milling of the whole kernel. The bran produced is mixed with a separated (mainly) endosperm protein fraction to produce animal feed. 4,171,383 describes dry milling of the whole kernel to produce an endosperm fraction, a germ fraction and a bran fraction. The endosperm fraction is then subjected to wet milling and separation of starch-rich and protein-rich fractions. The protein rich fraction is added to the bran to produce an animal feed. There is no description of a specific wet fractionation of the bran itself within either patent.

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WO 99/11672 patent application discloses a process that uses selective enzymes, such as acetyl xylan esterase and ferulic acid esterase, to both facilitate the removal of hemicellu-

lose from various plant materials and alter its degree of phenolic ester substitution. Despite the fact that functional hemicellulose with high solutility and gelling strength can be produced yields are rather low. In fact, the inventors reported a 3 and 6% yield of arabinoxylan ferulate (hemicellulose), when wheat bran was treated with acetyl xylan esterase for 90 and 180 min, respectively. Furthermore, the invention does not make any reference to the use of xylanases, or its combination with wet milling in order to overcome the low yields reported.

US-A- 5,308,618 discloses a process to extract soluble dietary fibre hemicelluloses from wheat bran by applying a heat pre-treatment in aqueous solution. This is followed by further processing such as filtration, salting out, dialysis, ultrafiltration, reverse osmosis, gel filtration and precipitation in order to remove contaminants from the hemicellulose fraction. The inventors make no claims with regards to the use of enzymes and production of products streams other than hemicellulose. Furthermore, the invention highlights the need of run costly procedures to remove contaminants, which were once present in the original wheat bran. The bran is extracted at high temperatures and pressures in water (180 - 200° C), producing a glucose rich dietary fibre component in the water phase. The process specifically targets the production of dietary fibre and is not really / strictly a fractionation procedure in that other products are largely ignored.

US-A-3,879,373 discloses a process to extract hemicelluloses from wheat bran by applying alkali treatment to dissolve hemicelluloses and other bran components followed by ethanol extraction to separate the hemicelluloses. Alkali (sodium hydroxide) extraction of hemicellulose has also been disclosed in US-A-5,174,998 as an intermediate step to produce controlled-release compositions containing the said alkali-extracted hemicellulose and an active substance. Similar alkali-extraction procedure is disclosed in US-A-4,927,649 to produce hemicellulose, which is then used in coating compositions containing insoluble dietary fibre.

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WO 00/04053 patent application describes a chemical process using alkaline peroxide treatment to produce high yields of light coloured gelling hemicelluloses from products derived from flour, husk or bran. Another chemical extraction process of hemicellulose from wheat bran has been disclosed in WO98/31713 patent application, whereby the inventors combine a washing procedure to remove the starch fraction followed by an alkaline treatment with sodium hydroxide to extract the hemicellulose from the starch-free raw material.

- It appears from above-described prior arts on alkali extraction of hemicellulose that this is an old, proven and effective way to yield high quantities of soluble hemicellulose with interesting functionalities such as gelling, dietary fibre and as an inert material for controlled-release compositions. The drawback of such technology is the associated problems of utilising chemicals. Firstly, chemicals eventually become contaminants in various product streams, and therefore require additional purification. This normally has significant cost implications. Secondly, innovative industrial processes based on chemical extraction are not always attractive from the marketing point of view, particularly in food applications.
- Production of insoluble dietary fibres from oats is disclosed in US-A- 5,023,103, which describes a chemical procedure (alkali and bleaching treatment) for the production of insoluble dietary fibre with high water holding capacity and non-gritty mouth feel. A water holding capacity of 6.9g water/g oat fibre has been reported.
- Other references have disclosed interesting processes for the extraction of proteins from cereal brans. US-A- 4,746,073 discloses a physical process to separate aleurone cell particles and pericarp-testa particles from commercial wheat bran. The process consists of milling the bran particles to a specific particle size distribution, electrostatically charge the said particles and then pass the said charged particles through a magnetic field, which separates aleurone from pericarp-testa particles. This is a rather different concept from the current invention, which is based upon the use of enzymes and wet milling. The separation is achieved by hammer-milling the bran and then subjecting the resultant particles to a

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physical separation regime. No wet processing is employed during the fractionation procedure described therein.

Waszczynskyj et al. (1981) have proven that protein extraction rate of alkali-treated full fat wheat bran can be increased from 30% up to 38.5% when it is preceded by polysaccharidase treatment. The above-mentioned figures are significantly lower than those described in the present invention whereby up to 60% protein extraction rates were achieved without using alkali treatment. Furthermore, US 5,622,738 discloses a method to extract soluble hemicelluloses, for use as a source of dietary fibre, from various fibrous materials including cereal brans using alkali digestion followed by xylamuse treatment. As in other prior arts, the inventors made use of alkali digestion to improve extraction rates. Additionally, the residence time for the enzymatic treatment was rather long (3 to 96h), which makes the process not very attractive from the production cost point of view.

It is clear that none of the above-mentioned prior arts have succeeded to simultaneously yield alcurone proteins, oligosaccharides and hemicelluloses, and yet produce insoluble dictary fibre from previously cleaned cereal bran, i.e. substantially free of soluble components, using xylanases in combination with wet milling.

Furthermore, the current invention has arrived at an industrial process, which enables one to extract high amounts of such components (up to 50%) without using alkali digestion.

The main objectives of this invention were to:

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- 25 1. Arrive at an efficient and cost effective industrial wet process to extract and yield hemicellulose, oligosaccharides, aleurone proteins and insoluble dietary fibre from cleaned cereal brans.
  - 2. Combine the use of xylanases treatment with wer milling to improve the efficiency of extraction and separation in an industrial process.
- 30 3. Ensure that the raw material contains the least amount of readily extractable components, hence solubles, so that contamination with the said solubles in the end products is kept to a minimum.

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4. The process is carried out preferably with food grade and non-genetically modified (non-GMO) xylanases in order to broaden the market opportunities for the end products.

# 5 DESCRIPTION OF THE PREFERRED EMBODIMENTS

Various methods of extraction and fractionation of hemicelluloses from cereal brans have been developed in the past. Equally, various methods for the extraction of valuable proteins and insoluble dietary fibre from cereal brans have been disclosed. The problem is that when one combines the use of commercial cereal brans, which contain large quantities of soluble components such as starch, soluble proteins, pentosans, oils, etc, and a simple extraction process such solubles eventually become contaminants of the main product streams, and therefore have to be removed. This is a costly procedure and in many cases jeopardizes the market value of the non-hemicellulose components.

**EXAMPLE 1** 

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Wheat bran produced from short milling (SMB) and conventional milling (CMB) processes were used in this trial. Bran sample of 25 kg was transferred to a mixing tank and sequentially hydrolysed at temperatures varying from 70 °C at the first stage with α-amylase to 60 °C in the second stage with amyloglue besidase for a total hydrolysis time of 4 h. During this period the reaction mixture was intermittently wet milled to increase in surface area and dispersion of soluble components. The pH of the reaction mixture was set at neutral initially and then decreased down to 4.5 with acetic acid in the second stage. In addition of maximising the enzymatic activity the acidic pH allowed partial solubilization of the phytates present in the bran.

At the end of the enzymatic hydrolysis - wet milling step the enzymes contained in the reaction mixture were inactivated by wet heating through a heat exchange and quickly cooled down to room temperature.

The hydrolysed bran solution was then put through a two-phase decanter to separate the insoluble (fibre and aleurone fractions) from the soluble fraction.

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The soluble fraction was put through a separator so that the heavy phase containing mostly the germ components could be separated from the light phase containing mostly components from the remaining endosperm found in the bran. The light fraction, which was heavily contaminated with sugars, was put through an ultrafilter having a 50 kDa filter in order to separate low molecular weight sugars and a protein fraction with less sugar contamination from each other.

All soluble protein fractions, i.e. heavy and light phases, were blended together and finally processed through spray drying. The sugar fraction was concentrated by vacuum evaporation at mild temperature (40 to 60 °C) until a 75% sugar concentration was achieved. The fibre fraction was dried in a conventional laboratory oven, but in an industrial process this can be carried out by a number of different dryers, i.e. tumble drier, ring drier, fine grinder, etc.

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Average chemical composition of the brans and their respective fractions are shown below in Table 1.

# TABLE 1

Dry matter	Protein	Oil	Fibre	Ash	NNE***
90.8	15.7	4.1	45.4	5.5	29.3
he process		-1			
92.9	31.8	7.7	1.1	7.9	51.5
92.8	13.6	3.0	76.9	4.1	2.4
89.1	14.3	2.3	23.7	3.2	56.5
ne process				<del> </del>	
93.9	27.8	1.5	0.9	3.4	66.4
94.3	22.5	4.1	64,8	1.6	7.0
	90.8 he process 92.9 92.8 89.1 he process 93.9	90.8   15.7   he process   92.9   31.8   92.8   13.6     14.3       14.3     14.3     14.3     14.3     14.3     14.3     14.3       14.3       14.3       14.3       14.3       14.3	Dry matter   Protein   Oil	90.8 15.7 4.1 45.4 be process  92.9 31.8 7.7 1.1 92.8 13.6 3.0 76.9  89.1 14.3 2.3 23.7 ne process  93.9 27.8 1.5 0.9	Dry matter   Protein   Oil   Fibre   Ash

<sup>\*</sup> Conventional milling bran

<sup>\*\*</sup> Short milling bran

<sup>\*\*\*</sup> Non-nitrogen extracts

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# **EXAMPLE 2**

Wheat bran produced from conventional milling was subjected to enzymatic treatment and wet milling as described in Example 1. The hydrolysed bran was fractionated using a twophase decanter into an insoluble (combined fibre and aleurone) and a soluble fraction.

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The soluble fraction was fed into a separator for fractionation using centrifugal forces thus producing two phases. The germ-rich phase was wathed with water and fed again to the separator to remove the excess sugars and other light contaminants. The resulting protein fraction was kept as such or mixed with evaporated iquid whey on a 1:1 ratio (dry matter basis).

The endosperm-rich wheat fraction, which was heavily contaminated with sugars, was fed to an ultrafilter in order to separate low molecular weight sugars and a protein fraction with less sugar contamination.

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All soluble protein fractions, i.e. germ and endosperin-rich phases and the mixtures with whey, were spray dried separately. The sugar fraction was concentrated by vacuum evaporation at mild temperature (t = 60 °C) until a 75% sugar concentration was achieved. The fibre fraction was oven dried.

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The additional washing carried out on both germ-rich protein and fibre fraction was very effective to decrease the amount of light soluble continuinants from each fraction, and therefore increase the relative content of valuable components.

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Compositional data indicates that germ and endosperm-rich protein fractions have a different relative content of protein and oil. Protein and oil content from the former were 48.6 and 18.6%, respectively and those from the latter were 28.7% and 1.5%, respectively. The insoluble phase containing primarily the bran pericarp (fibre) and the aleurone proteins had 86.4% fibre and 12.6% protein. The chemical composition of the germ-rich phase whey mix was 31.5% protein, 9.8% oil and 37% lactose.

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A further important observation was that the spray died germ-rich fraction containing 18.6% oil was substantially more resistant to oxidation (rancidification) compared to the

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original wheat bran. The original wheat bran started tetting rancid after 3 weeks of storage. Despite the fact that no exogenous anti-oxidants were added to the germ-rich fraction it only started going off after 12 weeks of storage.

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## EXAMPLE 3

Previous examples illustrate the use of starch-hydrolysing enzymes and wet milling followed by various separation steps in order to yield both protein, sugar and fibre fractions, the latter still containing substantially high amounts of alcurone proteins. It could be of interest for same applications to separate, at least parily, the aleurone proteins from the bran pericarp (fibre) and recover such proteins in the same fraction as the endosperm-rich fraction for instance.

A trial was set up in the same way as described in EHAMPLE 2, except that a cocktail of polysaccharidases containing both high cellulase and xylanase activities was added together with the amyloglucosidases, and let to work for 3 h. Temperature and pH conditions were kept unchanged. The resulting reaction mixture was further treated exactly as described in EXAMPLE 2.

The inclusion of polysaccharidase during the hydrollesis step had a positive effect with 20 regards to aleurone protein extraction and protein responsery as measured by the mass balance and protein content. The protein content in the endosperm-rich fraction increased from 28.7% (without polysaccharidases) to 34.7% (with nolysaccharidases) and the overall protein recovery was increased by 35% when polysadcharidase was added.

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# EXAMPLE 4

The colour of protein ingredients can be of importance particularly in some food and feed applications. Milk products such as caseinates, whey powder and whey protein concentrate have a light colour and soy protein concentrate have a light brown colour. These products are the main ingredients in high value feeds such as calf milk replacer. But, in same food applications such as sausage and hamburger despite the fact the inclusion level is much lower, colour can still play an important role in the product acceptability.

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The technical feasibility of bleaching the germ-rich fraction was assessed by two means. 1. Solely alkali and hydrogen peroxide bleaching, and 2. Alkali-free peroxidase and hydrogen peroxide bleaching.

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1. 10 (ten) g samples of germ-rich fraction were included in 1 L beakers containing 100 ml water. Samples were dispersed with stirring and ca. 1.25ml NaOH added until pH 12 was reached. Solutions were warmed at 50 °C and 3.5, 5 and 10 ml of 30% H<sub>2</sub>O<sub>2</sub> were added to different flasks to provide uptake levels 10, 15 and 36% H<sub>2</sub>O<sub>2</sub> on weight basis of germ-rich fraction. Mixtures were stirred for 1 h and neutralise with acetic acid.

Full bleaching was achieved with 15 and 30% H<sub>2</sub>O<sub>2</sub> Sample treated with 10% H<sub>2</sub>O<sub>2</sub> was only partly bleached. All alkali bleached samples became darker with drying.

- 2. 10 (ten) g sample of germ-rich fraction was incubated in 1 L beaker containing 100 ml water. Samples were dispersed with stirring and ca. 0.25 ml NS 51004 Novozymes per-oxidase was added. Solution was warmed at 50 °C and 3.5 of 30% H<sub>2</sub>O<sub>2</sub> was added to the flask, i.e. 10% H<sub>2</sub>O<sub>2</sub> on weight basis of germ-rich fraction, and the mixture stirred for 2 hrs.
- The peroxidase hydrogen peroxide bleaching was effective, consumed less chemicals and no darkening of the sample was observed after drying.

By utilising previously cleaned bran as the preferred raw material, the inventors have overcome many important production constraints and created interesting opportunities to extract and separate new components from cereal brans. Furthermore, the inventors have developed a simple method in which wet milling is combined with enzymatic treatment using food grade commercial xylanases, and cheap industrial separation processes.

Essentially, this invention allows one to economically produce proteins derived

substantially from the aleurone cells, hemicellulose, oligosaccharide and insoluble dietary
fibre.

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**EXAMPLE 5** 

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Laboratory scale trials were carried out on cleaned wheat bran to test extraction rates using xylanase treatment. The cleaned wheat bran used as a raw material contained less than 1% starch and at least 50% and 70% of the protein and of, respectively, originally found in the

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starting material had been removed.

Ten g cleaned bran were incubated in 150 ml water, the pH adjusted to 5.5 with acetic acid and an enzyme cocktail containing pentosanase and remicellulase activities was added at the following concentrations: 0 (control), 0.1, 0.25, 05, 1 and 2% (w/w basis). Reaction mixtures were kept at 40°C for 120 min. The treatment was terminated by inactivating the enzymes at 80°C for 30 min.

Results indicated relatively high extraction rates compared to the control treatment (no enzyme added) despite the amount of enzyme used. Extraction rates of 3.1, 32.0, 32.8, 33.1, 33.8 and 34.2%, respectively, were obtained from control, 0.1, 0.25, 0.5, 1 and 2% treatments, respectively.

#### **EXAMPLE 6**

A similar trial to that described above was carried out with a purified endo 1,4-beta 20 xylanase (pentosanase) at two levels of inclusion: 0.25 and 0.5% (w/w basis).

Extraction rates were also high, and increased from \$11 (control treatment - no enzyme) to 28.6 and 26.1%, when 0.25 and 0.5% pentosanases were added, respectively.

**EXAMPLE 7** 

Cleaned bran with the same specification as described in Examples 5 and 6 was used in a large-scale trial. The objective was to validate a process using standard industrial equipments, quantify process parameters, determine extraction rates of the various fractions, and ultimately characterize the end products.

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Cleaned bran (80 kg) was incubated in hydrolysis tartics containing 500 l water. The pH was adjusted to 5.5 and a purified endo 1,4-beta xylanase (pentosanase) was added at 0.5% (w/w basis). The reaction mixture was continuously stirred and intermittently wet milled while kept at 40°C for 90 min. The hydrolysis/wet milling reatment was terminated by heating up the reaction mixture to 90°C for 2 min in a hear exchange device.

The inactivated hydrolysate was pumped through a commercial two-phase decanter where the insoluble phase (insoluble dietary fibre) was separated from the solubles. The insoluble phase was dried and further milled in a commercial fine grinder using indirect heat.

The solubles were then pumped through another two phase decanter where a heavy phase (aleurone protein-rich fraction) was separated from a light phase containing the extracted hemicellulose fraction in the form of both soluble hemicelluloses and oligosaccharides. The protein-rich phase was spray dried.

The hemicellulose fraction was further separated by size exclusion using an ultrafiltration unit whereby the large molecular size fraction (soluble hemicellulose) was separated from the small molecular size fraction (oligosaccharides and sugars). The resulting fractions were further processed by spray drying into a fine powder or alternatively evaporating the excess water until 25% water content was achieved.

The following yields of insoluble dietary fibre, hemiqellulose, oligosaccharides and proteinrich fractions were obtained from cleaned wheat brank 51.0, 26.1, 17.3 and 7.7%, respectively.

# EXAMPLE 8

An insoluble fibre fraction extracted according to the procedure described in Example 7 was characterized with focus on its potential use as a source of dietary fibre and texturizer in food applications.

Typical composition was as following: dry matter 9\$1%, cell wall components 75%, protein 11%, soluble sugars 3% (of which at least 75% is glucose), fat 4% and minerals 1.5%.

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The water holding capacity (WHC), of primary importance when assessing the usefulness of insoluble dietary fibres, of the above-described product was 8.6g water/g sample on dry basis. For comparison purposes the WHC of wheat bran in the range of 3.5g/g and that of cleaned wheat bran is 7.5g/g. This indicates the improved water absorption of the fibre after cell wall components have been partly removed. Other commercial dietary fibres extracted from wheat straw and sugar beet have WHC of 6.3 and 7.9g/g, respectively.

## **EXAMPLE 9**

The protein fraction, which contains substantial amounts of aleurone proteins, produced as described in Example 7, have a very interesting chemical composition, functionality and is an ideal raw material for further processing.

A typical composition of the protein fraction is: dry matter 98%, protein 40%, sugar 3%, fat
15 18%, non-sugar carbohydrates 32% and minerals 5%

In order to determine the effect of protease treatment on the functionalities of the protein fraction, a protein sample was subjected to a mild protease treatment and the samples analysed for dry matter and protein solubility, emulsifying capacity and emulsifying stability.

The results are shown in Table 2, and clearly indicate the possibilities to further improve some important functionalities of the protein fraction

# 25 **TABLE 2**

	Protein fraction	Protein fraction treated	
Parameters analysed		with protease	
Dry matter solubility (%)	19.7	38.1	
Protein solubility (%)	18.4	55.5	
Emulsifying capacity (%)	52.5	90.6	
Emulsifying stability (%)	47.5	86.0	

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# EXAMPLE 5

Amongst the various end-uses of the germ-rich fraction of EXAMPLES 1-4 one could describe meat products such as hamburgers, sausages and meatballs. In such end-uses germrich fraction could replace meat, soy protein concentrate and isolate, but also milk casein and caseinates, to mention just a few. It is therefore important to test the overall performance of the germ-rich fraction with regards of emulsifying and binding capacity, taste, etc.

A trial set up to test the feasibility of incorporating various germ-rich fractions extracted from wheat bran into a traditional meat ball recipe consisted of meat, garlic, premix and 10 water.

The following spray dried fractions were tested:

- Germ-rich fraction extracted from short milling wheat bran (1) 15
  - Germ-rich fraction extracted from conventional milling wheat bran (II)
  - 1:1 mix of whey and II, on dry matter basis (III)

Meatball recipes were tested without germ-rich fraction (control recipe) or with 2.5% inclusion of samples I, II or III. Meatballs were analysed for weight loss, taste, texture and 20 colour after frying.

The results are described in the table 3 below.

# TABLE 3

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Recipe tested	Weight loss after frying (%)	Colour	Texture
Control (meat, garlic, premix and water)	23.4	Reference	Reference
Control + 2.5% of I	21.3	Slightly darker	Slightly tougher
Control + 2.5% of II	20.8	Similar	Similar
Control + 2,5% of III	18.0	Similar	Slightly more tender

The overall conclusion was that the samples performed well as additives in a meat ball recipe, and were particularly interesting as they all decreased the weight loss after frying.

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# END-USES

## Germ-rich fraction

The high protein content of the germ-rich fractions makes it an ideal substitute for existing expensive proteins from animal and vegetable origin. Additionally, the germ-rich fraction because of the nature of its protein, the presence of high quality oil and phospholipids also exhibit interesting functionalities such as emulsification, texture and binding.

One can list, as examples, the following existing products, which can be replaced by the germ-rich fraction in the food industry:

Animal protein: casein and caseinates, plasma protein and egg white Vegetable proteins: soy protein concentrates and isolates, texturized soy, hydrolysed gluten and potato protein,

Generally, the above products can be used as meat extenders and texturizer ingredients in hamburger, sausage, and meat balls production to mention a few. Or, as a casein replacer in the production of sausage, spreads, dressings, etc.

In the feed industry, the germ-rich fraction is an ideal ingredient for high value feeds such as calf milk replacer; starter feeds for calves, piglets and chicks, fish feeds and pet food. In such applications it can substantially replace the use of soy proteins (texturized soy, concentrate and isolate), potato protein, hydrolysed guten, high quality fish meal, plasma protein, and dry milk products such whey protein concentrate, whey and skimmed milk.

# Endosperm-rich fraction

The endosperm-rich fraction can be used in the food industry primarily as a gluten replacer, but also to partly substitute soy proteins in the production of various meat and vegetable products. In feed applications, it can partly replace gluten, soy and milk proteins as an ingredient to calf milk replacer, piglet started feed and fish feed.

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# Fibre fraction

The fibre fraction consisted of pericarp and alcurone can be used as an interesting source of gluten- and phytate-free fibre source to replace conventional cereal brans. The main enduses as food would be in the baking industry and breakfast cereal.

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One preferred embodiment of a plant for carrying out the invention is shown in the attached drawing,

wherein FIG. 1 a set-up for carrying out the process of the invention;

FIG. 2 shows a set-up for carrying out a preferred embodiment of the invention; and

10 FIG. 3 shows the fractionation of the bran in an over-view.

In FIG. 1, 1 denotes a suspension and hydrolysis vessel 1 and connected to a wet mill 2 which by its milling action increases the active surface of he hydrolysis. The slurry is allowed to pass the wet mill 2, 1 to 3 times. The sluthy is then transferred to a 2phase decanter 6 via a heat exchanger 3, which decanter 6 separates the bran and liquid (water and protein) phases. The bran having a dry matter content of about 40%, is then further washed once using water in a suspension vessel 4, and is allowed to pass a second decanter 6, again. Then a pure bran obtained has a dry matter content of 95% after drying in a ring drier 10. The collected liquid phases, having a dry matter content of 5%, are transferred to a separator 7, via a mixing tank 5, in which separator 7 insoluble protein is separated off. The liquid phase from the separator 7 having a dry matter content of 2%, and comprising water and soluble proteins is allowed to glass an ultra filter 8 having a molecular cut of 50 kD. Depending on different requirements this cut can vary between 20 and 100 kD. After the ultra filter 8 a liquid phase is obtained which is evaporated in an evaporator 9, and concentrated to a syrup, having the dry matter content of 75%. The concentrate of the ultra filtration is either isolated as such, or added to the fraction of insoluble proteins, which latter has a dry matter content of 9.5%. The protein-spgar fraction obtained is spray-dried in a spray drier 11 to provide a concentrated, dried projein-sugar fraction, and the protein-oil

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One preferred embodiment of a plant for carrying out the invention related to clean bran separation is shown in the attached drawing, FIG. 2 wherein 1 denotes a suspension and hydrolysis vessel to which a wet mill 2 is connected. The reaction mixture is intermittently

fraction is dried in a spray-drier 11 to provide a concentrated, dried protein-oil fraction.

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pumped through the wet mill 2 (from 1 to 3 times). The hydrolyzate is then inactivated in a heat exchange 3 and transferred to a two-phase decanter 4, which decanter 4 separates the insoluble (insoluble dietary fibre) from the soluble phase. The insoluble phase having a dry matter content of about 35% is dried to approximately 95% dry matter in a ring drier 5. The soluble phase, having a dry matter content of approximately 3%, is pumped through another two-phase decanter 7, via a holding tank 6, in which is two-phase decanter 7 protein-rich fraction is separated off. The protein-rich fraction is then dried to about 95% dry matter in spray drier 8. The soluble (liquid) phase from the two-phase decanter 7, having a dry matter content of approximately 3%, and comprising water and primarily soluble hemicellulose, oligosaccharides and sugar is allowed to pass an ultra filter 9 having a molecular cut of 20 kD. Depending on different requirements this cut can vary between 10 and 50 kD. The retentate (fraction retained in the ultrafilter) from ultrafilter 9 is evaporated to a syrup concentration of at least 75% solids in an evaporator 10 or alternatively in a spray drier such as 8. And, the permeate (fraction not retained in the ultrafilter) from ultrafilter 9 is preferably evaporated to a syrup concentration of at least 75% solids in an evaporator 11.

# Applications

## Insoluble dietary Fibre

This is the remaining fibre after the soluble components and a proportion of hemicellulose has been removed from rye bran. The insoluble dietary fibre is a cleaned cereal fibre containing low levels of phytic acid. Because the fibre has already been partially "digested" enzymatically, many beneficial compounds derived from the cell wall are available to the gut for absorption.

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The insoluble dietary fibre has high water binding capacity, i.e. typically 100% higher than that of wheat bran. This provides increased gut transit (digesta flow). The remaining pentosans are more accessible to the gut wall (choles erol reducing) due to the fractionation process. Because of the increased availability of light type materials and other antioxidants within the fibre, various health benefits can be claimed. Specifically the lighans and polyphenolics are known to mimic estrogens (female hormones), and more recently have been found to help preventing various types of cancers. This has been verified for rye products.

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The insoluble dietary fibre is very useful as a food additive as a water binding and texturising ingredient and is good for the gut as a distary supplement. Specifically the high water binding capacity and beneficial effect on bowel function makes it an interesting product for the biomedical market.

Additionally the insoluble dietary fibre is also a good raw material for the further extraction (enzymatically) of lignins, ferulic acids, lignans etc which are natural antioxidants and potential anticancer agents. These can be used in many biomedical and "cosmetic pharmaceutical" applications such as lotions, creams and moisturizers. The ferulic acid is an effective UV absorber and as such can be used in a sunscreen.

Insoluble dietary fibres are rich in accessible lignand and residual pentosans / hemicelluloses. Bacteria present in the colon convert plant lignans to mammalian lignan, enterolactone, using hemicelluloses as a fermentation medium. These compounds mimic oestrogens and appear to have a tangible, demonstrable effect on the suppression of hormone related cancers, eg breast, ovarian and prograte cancers. Rye insoluble dietary fibre specifically contains the lignans Secoisolaricingsinol (SECO) and matairesinol (MAT) which are known precursors of enterolactone. The insoluble dietary fibre from wheat also contains these lignans, but the effect is not demonstrated in wheat. It is important to state 20 that in this fraction the lignans are supplied in an addessible form, as the cell wall is already partially enzymatically digested, along with their natural synergistic partners, the arabinoxylan hemicelluloses remaining on the fibre

A further health claim is the improved water holding capacity of the fibre as compared to 25 normal bran. This increases digesta flow and reduces gut transit times, with concomitant benefits to health.

# Aleurone Rich Protein

This is the protein that is derived from the alcurone cell layer and is both a functional and 30 nutritionally valuable material, rich in essential ampo acids.

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In the food business it would be used as a emulsifier, foam stabilizer and texturiser. In addition there is high potential as a protein supplement.

#### 5 Soluble Hemicellulose

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This is the major cell wall non-cellulose polysaccharde in rye bran. It can be produced with high molecular weight and high solubility (the combination of these two properties is the powerful aspect). Because the product is a pentosan (arabino-xylan) it is low calorie and beneficial for gut health. The product can be produced with or without ferulate side chains or free ferulic acid and other antioxidants and is a free flowing cream powder.

Due to its composition and high water binding capacity it is ideal to use as a thickener, gellant, stabilizer, soluble dietary fibre and fat replacer. As a thickener and gellant it is interesting in the food industry as an additive in sours, margarine, deserts, pâtés, sauces etc. As a stabilizer it is a cheaper alternative for modified starch (made from wheat, maize etc), modified cellulose, gums (guar gum and carrageenan gum), alginates (seaweed), gelatin (cheap but problem with BSE) and pectin (fruit peel & sugar beet). Finally, it has a big potential in drinks because it is an excellent source of soluble dietary fibre alongside its stabilizing properties.

It is possible to supply the pentosan with ferulate side chains, and in this form the substance will gel in combination with oxygen and enzyme. As such it is an interesting material, for example, for wound dressings as it will keep the skin in a hydrated state and therapeutic agents can be added.

From rye and wheat, this is almost exclusively arabino-xylan (pentosan) hemicellulose. This is readily fermented in the colon, is low calorie to the human and is reported to generate butyrate as a short chain fatty acid (SCFA) end product after fermentation. This is the most "healthy" SCFA according to recent studies as it is a preferred source of energy to epithelial cells lining the colon. The health benefits of adding an enriched, available source of arabinoxylan to the diet may therefore be far-reaching.

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The fraction is a perfect soluble dietary fibre, with all of the concomitant health benefits. Arabinoxylans are also thought to be excellent binding sites for secondary bile acids, as a consequence of the rigidity of parts of the molecular chain and the occurrence of relatively hydrophobic domains on the polymer. This is thought to reduce any potential carcinogenic effects.

In addition, the fraction contains ferulic ester side chains to a proportion of the polymer, with concomitant free radical stabilising and anti-oxidant properties.

It is important to emphasise that the arabinoxylan concentrated in this fraction is not normally available to the gut and colon if presented a part of a normal diet or even from conventional bran.

In oat, this fraction is rich in β-glucan with all of the documented beneficial effects of this 15 polysaccharide. There is a tangible benefit in supplying a concentrated β-glucan of this nature as the normal ingestion of oats does not supply sufficient material for the full effects to be realised. Purified \( \beta \)-glucan can be purchased but is very expensive because of the extensive purification regime. It is important to realise that this high purification is required to remove the chemicals utilised in the extraction process. It is suspected that natural 20 synergistic partner compounds are removed in such a process, whilst these materials should still be present in the present process fraction.

## Oligosaccharide Syrup

This is derived from the hemicellulose fraction and is a 100% soluble dietary fibre of low molecular weight and low viscosity. The oligosaccharide syrup can be produced with lignans, ferulic acid and other antioxidants and is extremely soluble and hygroscopic.

It has a high potential in the drinks industry as it has low viscosity, is a good source of dietary fibre and gives good mouth feel and texture.

In combination with glucose syrup it could be used as a sweetener and energy source for drinks, cereal bars etc. As it is rich in ferulic acid, pentosans and solubilised lignans, one

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can also claim the related health benefits. It is very important to supply lignans in the presence of pentosan oligomers if the full cancer prevention effect is to be expressed and realised: precisely the situation in this fraction.

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The combination of glucose syrup and oligosaccharide syrup is also ideal in applications where one requires increased dietary fibre content and increased water binding capacity without thickening.

The oligosaccharide syrup is the low molecular weight fraction of the solubilised 10 arabinoxylans along with other low molecular weight components solubilised from the cell wall. This includes dissolved lignin fragments, phenolic compounds such as ferulic acid and lignans. As with the insoluble dietary fibre, the presence of lignans with arabinoxylan gives rise to claims for cancer preventative roles for rye and wheat derived fractions. In this case, the arabinoxylans are present as oligomers and the lignans are very available in the syrup 15 with a potential high accessibility for the gut. This should increase the rate of conversion of the plant lignans to enterolactone with a potentially larger impact on cancer prevention.

Furthermore, the presence of high concentrations of bligomeric arabinoxylan provides a ready fermentation substrate for the production of beneficial SCFAs such as butyrate, with benefits as described for the hemicellulose fraction.

This fraction, especially in the case of rye, is probably the most interesting in the present context being an excellent source of arabinoxylans, lignans and phenolic antioxidants in very accessible forms along with relevant synergistic partner compounds.

#### Germ Rich Protein

The germ rich protein is one of the products that are produced from the soluble fraction. It is one of the most valuable products because it contains the rye germ oil. Typically, it contains 55% protein and 17-20% oil, the latter being rich in soly-unsaturated fatty acids and in beneficial materials for cholesterol control.

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The germ rich protein is a good functional protein and can also substitute soy protein in many applications. Its good texturising effects are very interesting for the meat industry (meatballs, hamburgers, pate etc.). Properties such as solubility and emulsification can be further improved by enzymatic hydrolysis.

The germ rich protein is of great interest as a functional food ingredient, especially in the case of rye, primarily because it contains the germ of (see below).

# 10 Cereal Germ Oil

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All of the germ oils isolated using the present process are interesting from a health point of view. All contain the vitamin E tocopherol complex along with tocotrienols, vitamin B and polyunsaturated fatty acids. The case of rye germ oil is especially significant, as it is one of nature's richest sources of β-sitosterol, a documented cholesterol inhibitor, alongside tocotrienol, a cholesterol burner. Furthermore, the form of the sterol in the oil is suitable for adsorption. The present process represents a commercial route to rye germ oil. A formulation in margarines and spreads is suggested as a first step to commercial utilisation.

#### Rye Germ Oil

The germ oil is derived from the germ rich protein and is a very high quality food grade oil and ingredient. It is extracted without using any solvent, and it contains no preservatives or additives. It is a good source of poly and mono unsaturated fat, has a good flavour, is rich in vitamin E and can be suspended easily. As a flavouring component it is good in wheat or rye based products (cereals, baking goods, biscuits etc.), deserts, ice creams etc. It can also be useful as an ingredient in fat and oil formulations, juices etc. with natural vitamin E.

Rye germ oil is particularly rich in naturally occurring β-situaterol, a cholesterol lowering compound and tocotrienol, a cholesterol "burner". These materials can be classified as "natural synergistic partners", an important factor in the functional food area. This massively increases the potential of the oil as a value added neutraceutical ingredient in foods such as margarines and spreads.

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Rye germ oil is also a good UV blocker and therefore together with ferulic acid it can be ideal as a component of a sun tan lotion. Its emulsifying properties make it very suitable as an emulsion stabilizer and as an emollient ingredient for skin creams. It should be mentioned that there is presently no reasonable scale commercial production of rye germ oil and the present process is the only realistic way to achieve this.

# **Endosperm Rich Protein**

This protein fraction is mainly derived from the residual endosperm proteins in rye bran. It contains 35-40% protein, much of which is highly soluble. It is also particularly rich in pentosans (soluble dietary fibre), has a high water holding capacity and has a light colour.

The emulsifying, water binding and foam stabilising properties are equivalent or better than those of other commercial proteins like caseinates, soy protein concentrate and modified wheat gluten. The endosperm rich protein is very suitable to be used as an ingredient in milk replacer formulae (both for humans and calves), sauces, mayonnaise, dressings etc.

Because it contains high amounts of pentosans and associated ferulic acid there are extra health and functional benefits. In the cosmetic industry the stabilising, emulsifying and water holding properties are ideal.

A combination of the endosperm rich protein and soluble hemicellulose is interesting in a number of food and biomedical applications, because of the emulsifying effect of the endosperm rich protein and the soluble pentosans and the thickening effects of the soluble hemicellulose.

The endosperm rich oil is rich in pentosan hemicelluloses, mainly arabinoxylans in rye and wheat, or  $\beta$ -glucans in the case of oat. The claimed health benefits are therefore as described already for these fractions.

# Glucose Syrup

This is the glucose produced from enzymatic degradation of residual starch of the bran and is a more pure product compared to molasses. As such it is ideal as a feedstock for industrial

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fermentation since it produces less waste products. Ethanol and citric acid industries are therefore ideal consumers of very large quantities of such a product. The production of single-cell protein for the feed and food markets can also be considered. Alternatively, it can be used as a sweetening syrup in the standard fathion.

## **Defatted Germ Rich Protein**

This is the protein that remains after the rye germ of has been extracted from the germ rich fraction and has at least 70% protein content (is in the same range as soy protein concentrates). It is also a good functional protein, has an extremely high fat binding capacity and can be easily upgraded enzymatically to increase solubility, emulsion and foam stabilisation properties.

The product is an excellent stabiliser for water in oil smulsions and is interesting as a meat texturiser or extender in sausages, burgers, patées etc. The defatted germ rich protein is a functional protein that can easily replace soya proteins and contains phospholipids, natural lecithins and glycolipids.

It has a high potential in cosmetic formulation as an emulsion stabiliser because it contains natural lecithins.

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# **CLAIMS**

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1. Process for the wet fractionation of cereal bran components,

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# characterized in

that bran is first subjected to a combination of enzymatic treatment with enzymes of the
group starch- and phytate-hydrolysing enzymes, and wet milling, followed by an optional
step of enzyme inactivation by wet heat treatment, and the next step whereby the insoluble
phase containing both pericarp and aleurone fractions are separated by centrifugal forces
from the aqueous phase containing germ and residual endosperm components to produce a
clean bran, and that the aqueous phase is further separated by centrifugal force into a germrich fraction and an endosperm-rich fraction, and that the proteins contained in the
endosperm-rich fraction are concentrated.

- 2. Process according to claim 1,
- wherein cereal brans are the fibrous-residue resulting from a primary grain milling, i.e. after the separation of the endosperm fraction, of wheat, nice, barley, oat, rye and triticale, and having variable chemical compositions, presence of anti-nutritive factors, and presence of various anatomical fractions, i.e. pericarp, germ, and residual endosperm.
  - 3. Process according to claim 1,
- wherein the combination of wet milling with enzymatic treatment is arranged to increase substrate accessibility thereby improving the overall hydrolysis performance and the subsequent separation of both insoluble and soluble fractions of varied density/solubility.
  - 4. Process according to claim 1,
- wherein the enzymatic treatment is accomplished by using a non-starch degrading enzyme in the form of a polysaccharidase of amylases and/or amyloglucosidases.
  - 5. Process according to claims 1-4,
    wherein a further enzymatic treatment is carried out using at least one non-starch degradable
    polysaccharidase in the form of cellulases, hemicellulases mainly xylanases, beta
    glucanases, and pectinases, and/or phytases.

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- 6. Process for the wet fractionation of cereal bran substantially free of soluble compounds according to claim 1,
- wherein such clean bran is subjected to a combination of enzymatic treatment with specific enzymes of the group xylanase under strictly controlled hydrolysis conditions, and intermittent wet milling, followed by an optional step of enzyme inactivation by wet heat treatment.
  - 7. Process according to claim 6,
- wherein the inactivated hydrolysate is then fractionated by centrifugal forces into an insoluble phase containing primarily cellulose, lignin, less accessible hemicellulose, residual aleurone cells and cell wall bound proteins, and an aqueous phase containing soluble hemicellulose, oligosaccharides, sugars and proteins, and that the aqueous phase is further separated by centrifugal force into protein-rich fraction and a carbohydrate-rich fraction, and that the carbohydrate-rich fraction is further separated by size exclusion technique into a hemicellulose-rich fraction (large molecular size) and an oligosaccharide-rich fraction (small molecular size).
  - 8. Process according to claims 6-7,

- wherein cereal bran substantially free of soluble compounds, both in water and/or non-polar solvents are derived from wheat, rice, barley, oat, rye or triticale.
  - 9. Process according to claims 6-8, wherein the combination of intermittent wet milling with enzymatic treatment is arranged to increase substrate accessibility to the cell wall degrading enzymes thereby improving the overall hydrolysis performance and the subsequent separation of the various fractions by density/solubility and molecular size.
  - 10. Process according to claims 6-9,
    wherein the enzymatic treatment is carried out using at least one non-starch degradable
    polysaccharidase in the form of cellulases, hemicellulases mainly xylanases, beta
    glucanases, and pectinases, and/or phytases.

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- 11. Process according to claim 10,
  wherein the enzymatic treatment is accomplished by using xylanases with high beta 1-4xylanase (pentosanase) activity.
- 12. Protein fraction derived substantially from the serm and produced according to claims 1-4,
  wherein the said fraction contains at least 40% protein and 10% oil on dry matter basis and exhibits an increased shelf life with regards to resistance to oxidation compared to the original bran, and that the said fraction contains less than 1% fibre, and it retains the emulsifying capacity of wheat germ.
  - 13. Protein fraction according to claim 12, wherein liquid whey is incorporated in to the said fraction at levels varying from 20 to 80% by weight on dry matter basis, and that the final mixture is dried.
  - 14. Protein fraction derived substantially from the residual endosperm and produced according to claims 1-4, wherein the said fraction contains at least 35% protein and 10% sugar and less than 2% oil and 1% fibre.
    - 15. Protein fraction according to claim 14, wherein liquid whey is incorporated in to the said fraction at levels varying from 20 to 80% by weight on dry matter basis, and that the final mixture is dried.
- 25 16. Fibre fraction produced according to claims 1-4 wherein the said fraction consists of cell wall components of bran (>85%) and alcurone proteins (>10%), and substantially free of gluten and starch.
  - 17. Sugar fraction produced according to claims 1-4 wherein the said fraction is originated primarily from the residual endosperm and it contains more than 65% sugars (such as glucose, maltose and malto-triose) on dry matter basis.
    - 18. Insoluble dietary fibre according to claim 16, used for recovery of lignans.

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- 19. Insoluble dietary fibre according to claims 6-7, used for recovery of lignans
- 20. Aleurone rich protein produced in accordance with claims 6-11.
- 21. Soluble hemicellulose produced in accordance with claim 6-11.
- 22. Oligosaccharide syrup produced in accordance with claim 6-11.
- 23. Germ oil produced in accordance with claims 1 5. 10
  - 24. Rye germ oil produced in accordance with claims 1-5.
  - 25. Defatted germ rich protein produced in accordance with claim 1-5.
  - 26. Use of a protein fraction, as described in claims 0-13, and 17, in feed and food applications to replace other protein products from vegetable and animal sources.
- 27. Use of a fibre fraction, as described in claim 16 18, 19, in feed and food applications to replace other insoluble fibrous products or as a raw material for further processing. 20
  - 28. Protein fraction derived substantially from the alturone cells and produced according to claims 6-11,
  - wherein the said fraction contains at least 40% protein and 15% oil, less than 1% insoluble fibre on dry matter basis, substantially free of gluted and starch and with a high emulsifying capacity.
    - 29. Protein fraction according to claim 28, wherein the fat can be optionally removed by convertional organic solvent extraction or preferably by supercritical carbon dioxide extraction to yield a defatted protein and an oil fraction.
    - 30. Protein fraction according to any of claims 10, 12, 28, and 29,

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wherein proteases are incorporated in to the said fraction in wet state and at controlled temperature and pH conditions, and the resulting projein hydrolysate has enhanced functionalities such as solubility, emulsifying and fdaming capacities.

- 31. Insoluble fibre fraction produced according to claims 6-11, wherein the said fraction consists primarily of cell wall components with a relative lower hemicellulose content compared to the original cleaned cereal bran, substantially free of gluten and starch (<1% on dry matter basis) and with a high water holding capacity (>8g water/g dry product).
- 32. Soluble hemicellulose fraction produced according to claims 6-11, wherein the said fraction consists primarily of high molecular weight hemicellulose of preferably above 20 kDa (>30%), which also contains proteins (<10%) and monosaccharides (<10%), and is substantially free of gluten and starch (<1% on dry matter basis).
  - 33. Soluble oligosaccharide fraction produced according to claims 5-9, wherein the said fraction consists primarily of low melecular weight hemicellulose sub-units of below about 20 kDa (>30%), which also contains proteins (<10%) and monosaccharides (<20%), and is substantially free of gluten and starch (<1% on dry matter basis).
  - 34. Use of a protein fraction, as described in claim 29-30, in feed and food applications to replace conventional protein ingredients and functional proteins both from vegetable and animal sources.
  - 35. Use of an insoluble fibre fraction, as described in claim 31, in feed and food applications as a water binder and texturizer to replace other insoluble fibres, or as a raw material for further processing to extract the remaining cellulose and lignin.
  - 36. Use of a soluble hemicellulose, as described in claim 32, in feed, food and chemical applications as a gellant, thickener, emulsifier and/or as a source of soluble dietary fibre, or as a raw material for further processing to obtain other functional hemicelluloses.

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- 37. Use of a soluble oligosaccharide, as described in claim 33, in feed and food applications as a functional soluble dietary fibre or low calorie sweetener.
- 5 38. Set up for carrying out the process according to claims 1-4, characterized in

that it comprises a hydrolysis vessel (1), a wet mill (2), a heat exchange for enzymatic inactivation (3), washing tanks (4), a mixing tank (5), a decanter (6), a separator (7), an ultra-filter (8), an evaporator (9), and optionally dryers (10).

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39. Set up for carrying out the process according to claims 6-11,

characterized in

that it comprises a hydrolysis vessel (1), a wet mill (2), a heat exchange for enzymatic inactivation (3), decanters (4 and 7), dryers (5 and 8), a holding tank (6), an ultra-filter (9) and evaporators (10 and 11).

40. Process according to claims 1-5, wherein the enzymatic treatment is carried out at a pH of 4 to 7.5 and at a temperature of from 50 to 90°C, at an enzymatic activity of 200 to 100 IU/g of substrate.

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41. Process according to claims 6-11,

wherein the enzymatic treatment is carried out at a pill of 4 to 7, preferably 4.5-5.5, and at a temperature of from 35 to 80°C, at an enzymatic activity of 200 to 1500 IU/g of substrate.

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# **ABSTRACT**

A process for the fractionation of cereal brans substantially free of soluble compounds is described. In particular, this invention describes a two step process, in which the said cleaned bran is subjected to a combination of enzymatic treatment with xylanases and wet milling, and a second step consisting of sequential centrifugation and ultrafiltration, which aims at physically separating the main fractions, i.e. insoluble phase (remaining cell wall components), protein-rich fraction, soluble hemicellulose and oligosaccharide. This invention also describes a process that maximizes the extraction rate of valuable cell wall components and aleurone cells from previously cleaned bran.

10 (FIG 1)

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